REMARKS

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References to Application

All references to the application herein are made with respect to the substitute specification, filed September 18, 2002 with Response A.

Information Disclosure Statement

A previously submitted information disclosure statement (submitted May 10, 2001) that the Examiner surmised was submitted before May 29, 2001 was resubmitted to the USPTO on January 13, 2003, along with proof of receipt of that submission in the form of a returned post card (dated May 14, 2001).

Amendments:

Drawings

As suggested by the Examiner in the Office Action on p. 2, Applicants have amended the figures to be consistent with the amendments made in the specification in Response A, filed September 18, 2002. Therefore, Figures 5, 6, 10a and 10b, 23, 24, 25c, 26c, 27a-27d, 28a-28d,

29a, 29b, and 30-36 have been amended to replace the terms "precision panel," "precision profile," "precision profiling" and "precision profiles" with "selected panel," "selected profile," selected profiling" and "selected profiles." The changes have been made to address inconsistencies between the substitute specification, file September 18, 2002, and the figures submitted with the application on March 29, 2001.

In addition, a number of figures have been amended to change Arabic letters to Greek letters in gene abbreviations used to label individual panels (see Figs. 11a-11b, 12a, 13a-13d, 14, 15, 16a-16b, 17, 18a-18e, 19a-19b, 21, 22, 23, 24, 25a-25c, 26a-26d, 27a-27d, 29a-29b, 30, 33, 34,35, and 36). Other figures have been amended to correct clear error which occurred as a result of transpositions from the draftsperson from the originals (see Figs. 11a-11b; 16a-16b; 18c-18e; 22; 29a-29b; and 35). Still others have been amended to flip the orientation of gene abbreviations 180° to make them consistent with all other figures (see Figs. 29a-29b and 30). Finally, Fig. 31 has been amended to correct a clear error in the dosage listed in the label for the figure – i.e. the incorrect amount of 100 mg/kg/day was amended to the correct amount of 400 mg/kg/day. Support for this amendment is found in the specification in Example 17 on p. 56, lines 31-32 ("Males rats were treated with 40mg/kg/day of clofibrate administered by mouth and the levels of gene expression were measured in liver tissue").

Applicants respectfully submit that no new matter has been added with these amendments and request that the amendments to Figures 5, 6, 10a -10b, 11a-11b, 12a, 13a-13d, 14, 15, 16a-16b, 17, 18a-18e, 19a-19b, 21, 22, 23, 24, 25a-25c, 26a-26d, 27a-27d, 28a-28d, 29a - 29b, and 30-36, submitted as Substitute Formal Drawings, be entered into the record.

Specification

Applicants have amended several paragraphs and one complete page in the specification,

to correct minor typographical errors in most cases, and to correct clear error in others. Thus, on page 1, the Technical Field section has been amended to correct the incorrect word "resent" in line 13 to the correct word "recent." Similarly, the last word of line 12 has been corrected from "of" to "or", the word "be," missing from lines 16 and 18 of page 25, has been added in both lines, and the incorrectly spelled word "Appled" in line 21 of page 45 has been corrected to read "Applied."

The entire page 46 has been replaced. Degree symbols, incorrectly inserted in lines 8, 26, 27, and 30 have been corrected; an Arabic B in line 31 has been replaced with the correct Greek letter β (b-actin amended to β-actin); and the unit of volume "mL" in lines 11, 12, 20, 21, 22, and 23 has been changed to "μL" in all instances except for the description of the "1.5 mL microcentrifuge tube" on lines 21-22 that was used in each experiment. Applicants respectfully submit that the "mL" units are clear error, since a 1.5 mL microcentrifuge tube cannot possibly hold 80 mL of total reaction volume for each sample (see line 20).

Other minor errors were also corrected, in attempts to correct all typographical errors at one time. Thus, missing degree symbols have been added in lines 31 and 33 on p. 49, and line 3 on p. 50, of Example 5. Similarly, an Arabic a was amended to a Greek α in line 24, p. 51 of example 8 (TNF-a amended to TNF-α).

Finally, bracketed text, meant as questions to the inventor in a draft version of this application, were either deleted and clarified, as in line 27 of Example 16("a subject [or in vitro]" was deleted, and "five difference cell lines" was added, support for which is clearly found in the figure legend of Figure 30 itself; or simply deleted, as was "[details]" from line 29 of page 56, Example 16.

Applicants respectfully submit that none of these changes represent addition, or deletion,

of new matter.

Claims

Support for new claims 180 – 184, which depend variously from claims 167 and 175, can be found in the specification as follows:

Claims 180-181: p. 10, line 8 through p. 11, line 4; and p. 23, lines 6-19.

Claim 182: p. 26, lines 4-15.

Claims 183 – 184: p. 26, lines 8-16.

Claim Rejections – 35 USC § 112, para. 1

THE PENDING CLAIMS AS AMENDED CONTAIN NO NEW MATTER

Claims 167 – 179 have been amended to address the 37 CFR § 112, para. 1 rejections cited by the Examiner on pp. 2-7 of the Office Action mailed December 24, 2002 ("the Office Action"). The bases for claims 167-174, claims 175-179, and new claims 180-189 are as follows:

Claims 167 and 175. Amended claims 167 and 175 require "deriving ... a profile data set ...; and in deriving ... obtaining such measure ... under conditions that are substantially reproducible ... and efficiencies of amplification for all constituents are substantially similar." Support for these claims is found in the specification on p. 23, lines 6-9 ("We have found that we can measure concentrations ... in a manner that is both highly precise and reproducible ... under the same conditions ... such concentration measurements are reproducible...") and p. 10, lines 14-15 ("... such measurement is performed ... under conditions wherein efficiencies of amplification for all constituents are substantially similar....."

Claims 168 and 176. Amended claims 168 and 176 require that " ... measurement conditions are reproducible so that such measure has a coefficient of variation ... less than approximately 3

percent." Support is found in the specification on p. 23, lines 8-9 ("... such concentration measurements are reproducible..."); lines 13-14 ("... we found an average coefficient of variation ... of less than 1 percent..."); lines 18-19 (" we found an average coefficient of less than 2 percent."); and lines 21-23 ("We have found it valuable ... to identify and eliminate data points ... that differ by a percentage greater, for example, than 3 %...").

Although the Examiner "found no references that discussed determining a coefficient of variation any lower or in the range of the claimed limitation of 3 %" (see Office Action, p. 6), Applicants respectfully submit that the above-cited disclosure of coefficients of less than 2 percent and less than 1 percent provides the support for a coefficient of variation of less than 3 %; moreover, in this context, the reference to related concept of elimination of data points outlying the 3% figures provides further support for the claimed limitations.

Claims 169 - 171. Amended claims 169-171 require "efficiencies of amplification of all constituents [that] differ by less than approximately [X]%."Support for the limitation wherein efficiencies of amplification "...differ by no more than 10 percent" (claim 169), "2 percent" (claim 170), or "1 percent" (claim 171) is found in the specification on p. 26, lines 10-11 ("... it is necessary that all constituents of the panels maintain a similar and limited range of primer template ratios ... and amplification efficiencies ...") and lines 13-15 for claim 169 ("We regard amplification efficiencies as being "substantially similar" ... if they differ by no more than approximately 10%"); and lines 15-16 for claims 170 and 171 ("... preferably they should differ by less than approximately 2% and more preferably by less than 1%").

Claims 172 and 177. These claims require "at least three constituents" in the panel. Support for this limitation is found in the specification on p.17, line 20 wherein the definition for what constitutes a panel specifically states that "A "panel" of genes is a set of genes including at least

two constituents." Applicants respectfully submit that a limitation for a panel of genes including "at least three constituents" falls within the definition of a panel being "a set of genes including at least two constituents." Thus, combined with support from original claim 53 (A method ... wherein the number of constituents in the panel is at least three but less than 100) and the support of original claim 56 (A method ...wherein the number of constituents in the panel is at least is at least [sic] six.) there is support for the broader concept of "at least three constituents" claimed in claims 172 and 177.

Claims 173 and 178. These claims require a panel with "fewer than approximately 500 constituents." This claim implicitly has a lower limit of two constituents because the term "panel" is defined to include "at least two constituents" (*see above*, and p. 17, line 20 of specification). Thus, the claims define a limitation of from 2 to 500 constituents. Additional support is found in the base claims from which these claims depend. Independent claims 167 and 175, require "... a profile data set ... including *a plurality* of members ... of constituents ...." *See* claims 167 and 175 above. A plurality of members is two or more members.

Therefore, Applicants respectfully submit that there is support for the broader concept in claims 173 and 178 for a panel having "fewer than approximately 500 constituents, given that claims 173 and 178 depend from claims 167 and 175, which require a plurality of constituents – i.e. at least two – and that the specification defines a panel has having "at least two" constituents on p. 17, line 20.

Claims 174 and 179. These claims require that "the biological condition being evaluated is with respect to a localized tissue ... and the sample is derived from tissue or fluid ... distinct from that of the localized tissue." As stated in Response A, filed September 18, 2002, support for this limitation is found on page 22. Specifically, the section on page 22 titled "Selected Panels"

(specifically lines 5-17) discusses how to select constituents for a panel that "directly or indirectly vary with a particular biological condition" stating that "... a selected panel is not necessarily selected according to an expected profile of gene expression in cells that directly respond to a biological effect. For example, gene expression associated with liver metabolism may be analyzed in a blood sample."

Applicants respectfully submit that the definition on p. 17, lines 29 *et seq* of a biological condition explicitly states that it "may include any aspect of the subject capable of being monitored for change in condition, such as health ... or mood." Therefore, in contrast to the assertions of the Office Action (see p. 4) that there is only support for the more narrow concept of original claims 31 and 32, e.g. "the condition is clinically manifested (claim 31) and the biological condition being a disease ... (claim 32)," the specification on p. 22 coupled with the definition for biological condition on p. 17 conclusively provides "support for the broader concept now claimed." *See Id.*

In light of the above arguments, Applicants respectfully submit that there is support in the specification for all the limitations in claims 165-179 and new claims 180-189 and therefore no new matter has been added.

THE PENDING CLAIMS AS AMENDED ARE ENABLED BY THE DISCLOSURE OF THE APPLICATION

Design of primer/probe sets

The polymerase chain reaction has been known since 1984, when Kary Mullis realized the importance of adding a second primer, complementary to the second strand of double-stranded DNA (dsDNA), to replication reactions previously containing only a single primer complementary to the first strand of the dsDNA. As stated by Dr. Tryon "With this single invention, all the biological conditions necessary to permit the self-replication of DNA were met

and the result was multiple (many to billions; the amount dependent on the thermodynamic conditions), double-stranded copies of the original or template DNA." See Declaration, para. 5.

Since this revolutionary discovery, those skilled in the art have long used a variety of resources to help in designing primer/probe sets that meet the desired goals of the individual laboratory. For example, the present application cites Molecular Methods for Virus Detection (see p. 45, lines 14-15); the Taqman™ PCR Reagent Kit Protocol (*id.*, lines 16-18); and Current Opinion in Biotechnology (*id.*, lines 23-27) as resources to use in designing primers under section (b), "Amplification Strategies" of Example 1. Additional information in these cited references would be available and helpful to those skilled in the art, "for example, Detection of Viral Pathogens Using PCR Amplification by B. J. McCreedy in *Molecular Methods for Virus Detection, Primer Selection* D.F. Wiebauk and D. H. Farkas Eds. (1995), Academic Press, San Diego, pages 181-182. Declaration, para. 13, (2).

According to Dr. Tryon, "Perhaps the most authoritative collection of methods and analysis concerning the quantitative aspects and underlying design methods of PCR and RT-PCR known to me in 1999, and through the present, is found in the molecular medicine series entitled *Quantitative RT-PCR*; Paul D. Siebert, Chapter 4, pp. 61-86; *In*: Methods in Molecular Medicine, Volume 26; Quantitative PCR Protocols; B. Kochanowski and U. Reischl (Eds.) Humana Press; Totowa, New Jersey; 1999; ISBN 0-89603-5218-2 On p. 62 of that reference, Siebert identifies the known (as of 1998-1999) factors that affect the efficiency of amplification (*sensu stricto*, i.e., as known in the art, not as applied by the examiner)." Declaration, para. 12, and "Siebert Excerpt" attached as Exhibit B).

Those known factors are 1) the sequence being amplified; 2) the sequence of the primers; 3) the length of the sequence being amplified; and 4) impurities in the sample. Although the

Office Action holds that "The specification provides no guidance as to how the primer/probe sets are designed to give the desired results (see p. 5, last para.), as shown by Dr. Tryon,

> each of the known factors affecting amplification efficiency identified by Professor Siebert is addressed specifically in the application.
>
> (1) **The sequence to be amplified:**
> "The primer probe should not amplify genomic DNA or transcripts or cDNA from related but biologically irrelevant loci." (Specification, p. 26, line 30)
>
> (2) **The sequence of the primers:**
> "The reverse primer should be complementary to the coding DNA strand; located across an intron-exon junction, with not more than three bases of the three-prime end of the reverse primer complementary to the proximal exon." (Specification, p. 26, line 25)
>
> (3) **The length of the sequence being amplified:**
>
> "The primer probe should amplify cDNA of less than 110 bases in length." (Specification: p. 26, line 29)
>
> (4) **Impurities in the sample:**
> Preparation of the sample is disclosed at length in the application under example 1 on page 43, line 25 through page 44 line 31 and in the reference material cited in the application." Declaration, para. 13.

Further, as to the sequence of specific primers, those skilled in the art have long known how to design primers to meet experimental objectives, so such sequences are not essential for the disclosure to allow someone skilled in the art to practice the invention. As explained by Dr. Tryon, "... primer design is thoroughly covered in operator manuals supplied by PCR-related equipment manufacturers and reagent suppliers, in thousands of journal articles and many popular PCR protocol manuals including: *Amplification of Genomic DNA: Primer Selection,* pages 15-16, in, PCR Protocols: A Guide to Methods and Applications; M.A. Innis, D.H. Gelfand, J.J. Sninsky and T. J. White (Eds.); 1990; Academic Press; San Diego, CA; ISBN: 0-12-372180-6". Declaration, para. 14.

Most importantly, it may seem at first glance that a potentially infinite number of primer sequences may be experimentally determined that would satisfy the requirements of the presently claimed invention, or that a theoretically infinite number would have to be tested to determine the specific primer sequences which work. In reality, there are only a few sequences that could *potentially* satisfy the requirements of the presently claimed invention, and even fewer which *actually will* satisfy the requirements. And one skilled in the art would know and understand this by reading the specification and noting the requirements of primer design – i.e., only those which meet the design criteria outlined on p. 26 are even considered for experimental testing. Then, experimental validation is done of the actual primer/probe sets which yield amplification efficiencies with the very low coefficients of variation cited in the specification that will satisfy the requirements for primer probe eventually selected for practice of the claimed invention, keeping in mind the 4th factor cited by Dr. Siebert – impurities in the sample to aid in achieving the required amplification efficiencies. As elaborated by Dr. Tryon:

> Depending on the length of the genomic target and the exon (coding region) complexity of the investigational locus, it may be true that a large number of primer sequences *could* meet the criteria established in the application. However, that observation is true for nearly every known primer design or selection scheme, *in silico* or otherwise. As described in the application, using the described design criteria for primer/probe design, our laboratory actually only initially identifies a *small* number of primer sets meeting these design criteria and we then experimentally test that small number to identify primer-probe sets that also meet the highly strict amplification efficiencies required to practice the invention and detailed in the application. Actual experimental verification of the amplification efficiency is essential to the practice of the invention since (1) it is required for the calibrated nature of the selected panel and (2) the calculated amplification efficiencies provided by all primer design programs in common use today and in the past rarely correlate well with actual results. Declaration, para. 15.

In spite of such guidance in the application for how to initially select the primer/probe

sets to be tested experimentally, and in spite of the wealth of knowledge generally available to one of ordinary skill in the art, the Office Action holds that because the inventors "do not provide the particular sequences of interest nor does the specification provide the primer/probes sets specific for any or all of these genes that result in the claimed specificity and efficiencies of amplification" (see Office Action, p. 6, first partial para.) that "one of ordinary skill in the art would be unable to reproduce any of the experiments presented in the figures as insufficient information is provided in the specification to do so." *Id.*

According to Dr. Tryon, this "*is not true* in the Applicants' experience. On some occasions in the Applicants' laboratory separate individuals, designing primer-probe sets for the same locus, have independently arrived at the same primer-probe combination using the rules identified in the specification and common skill." Declaration, para. 16. It is not necessary for one skilled in the art to have the specific sequences of the primer/probe sets to practice the presently claimed invention, because, in addition to the wealth of readily available knowledge in the field for one skilled in the art in how to design primer-probe sets, the presently claimed invention does not require specific sequences for the primer-probe sets, merely that the primer/probe sets are able to provide specificity and a particular amplification efficiency. "*A priori*, there is no necessity for a single, sequence-specific solution to the primer-probe requirement. In some case there may only be a single, unique solution. But again, depending on the length of the target sequence and the exon complexity, there may exist multiple combinations that meet the requirements of the invention." Declaration, para. 17.

Even in the situations where multiple solutions (primer-probe sets) may exist, it is only necessary that one or a few be identified, not all those possible, but even if all possible were identified, that number will be quite small. As stated by Dr. Tryon, "despite the "class" rather

than sequence-specific solution to the primer-probe requirement, there exists only a very small number of primer-probe combinations that fulfill the requirements of the invention. The smallest targets may only have one solution (plus its mirror strand equivalent). The longest targets may have perhaps 10 or fewer." Declaration, para. 18.

Applicants also respectfully draw the Examiner's attention to the Results section on p. 110 of the prior art reference cited in the Office Action (see "Quantitative RT-PCR Measurement of Human Cytochrome P-450s: Application to Drug Induction Studies," Rodriguez-Antona et al., *Arch Biochem Biophys*, (2000), **376**, pp 109-116), where Rodriguez-Antona et al. discuss how to design specific primers. Their protocol for primer-probe design is generally analogous to that provided in the instant application (although they fail to anticipate or suggest the concepts herein of using primer-probe design to control amplification efficiency and other PCR parameters). First, Rodriguez-Antona et al. use primer analysis software to design and assess potential primer sequences – i.e., knowledge readily available in the art. Next they describe how the "primers were selected in order to meet three [sic] requirements: (a) ..., (b) ..., (c) ..., and (d)....,[which are distinct from those disclosed in the application]" just as Applicants describe on p. 26, lines 25-31 of the instant specification how the primer-probe sets are designed and selected, including a set of three features to be associated with the selected primer-probes. Lastly, Rodriguez-Antona et al. verify the suitability of the primers they have designed and selected experimentally (see Rodriguez-Antona et al., p. 110, col. 2, final para.) according to their criteria, just as Applicants state on p. 26, lines 18-24 of the specification that "In practice, we run tests to assure that these conditions [for Applicants' distinct criteria] are satisfied ... we still find experimental validation is useful."

Applicants respectfully submit that such instructions, whether in Rodriguez-Antona et al.

or the instant specification, are enabling for designing and selecting primer-probe sets according to the criteria of Rodriguez-Antona et al. or the criteria of the instant claimed invention. It is known in the art to provide a set of primer-probe criteria, and then to develop primer-probe sets that may function according to whatever criteria have been provided; it is within the art to verify experimentally that the criteria have been satisfied. To similar effect, see Siebert et al., Exhibit B to Declaration, pp. 62-63 (amplification in PCR as a subject for experimental determination owing to influence of cited factors). Indeed, it is a commonplace in design of primer-probe sets, using software such as "Primer Design" (available from Applied Biosystems, Gloucester, CA), that when design criteria are specified for primers and probes, usually a substantial number of alternative selections are the result. See Declaration, para. 15, generally.

Providing such instructions is analogous to someone facilitating a stranger getting from one location to a desired location when the stranger has never been to the desired location. Actually taking the stranger to the desired location is one effective way to ensure that the stranger arrives at the desired location, akin to actually providing primer sequences in a reference disclosing how to design and select primer sequences for a desired objective. An equally effective way to get the stranger to the desired location is to provide the stranger with verbal/written instructions and a map. The map is the knowledge generally available in the art, and the verbal/written instructions are the required parameters that must be met when designing and selecting the primer probe sets. Experimental verification would be akin to cell-phone or telephone verification that the stranger actually arrived (or the stranger stopping to make sure a wrong turn was not taken so adjustments can be made accordingly). Applicants respectfully submit that the instant specification is enabled for the design and selection of primer-probe sets and that it is not necessary to actually list the sequences, just as it is not necessary to actually

take someone to a destination to ensure that the person gets there.

In addition, Applicants have "identified at least one other controllable factor affecting amplification efficiency." Declaration, para. 19. As described on p. 26, line 7, it is necessary to maintain a "... limited range of primer template ratios (for example, within a 10-fold range)..." Thus, as stated by Dr. Tryon, "Primer template ratios ... were found by us to affect the routinely achievable levels of amplification efficiency." *Id.* Because such ratios similarly affected all primer sets used, independent of target sequence, Dr. Tryon's laboratory found that, in their experience, the "... acceptable ratio varies over many fold depending on reaction temperatures, salt concentrations and other parameters. However, as disclosed in the application, we found the selected panel to be uniquely informative if and only if a single set of such reaction conditions were maintained for all assayed loci ... That is, a single set of reaction conditions is applied to all constituents of the selected panel, resulting in a calibrated profile of gene expression." Declaration, para. 20. (As discussed in further detail below, this systemic approach, reflected in the claims, is unique and important.)

The Office Action states that "None of the examples appears to provide evidence demonstrating that the limitations of claims 168-171 and [sic] achieved for any panel or sample. It is noted for example, that Erlander et al. (WO 00.28092) at page 4 shows coefficients of variation on the order of 15% or greater. The examiner found no references that discussed determining a coefficient of variation any lower or in the range of the claimed limitation of 3%." *See* Office Action, p. 6.

As a preliminary matter, it should be observed that percentage figures are pertinent to two related contexts: first, the reproducibility of measurement conditions (expressed as percent coefficients of variation, see application, p. 23, lines 13-15); and second, efficiencies of

amplification used conducting measurements (see application, p. 26, lines 4-24 and following). It is by controlling amplification efficiencies, among other parameters, that reproducibility standards are achieved.

Accordingly, there are actually three distinct points raised in the above quote from the Examiner that must be addressed. First, regarding lack of examples demonstrating the limitations of claims 168-171 (amplification efficiencies that differ by no more than 10, 2, or 1%), Applicants respectfully submit that all of the examples demonstrate the claimed limitations. As stated in the specification on p. 23, lines 4 through 20 "We have found that we can measure concentrations of constituents in *selected panels* in a manner that is both highly precise and reproducible .... Over a total of 900 constituent assays, with each assay conducted in quadruplicate, *we found* an average coefficient of variation ... of less than 1 percent for each assay" (emphasis added). The Office Action states again on p. 7, lines 5-6 that "None of the examples appears to provide evidence demonstrating the limitations of claims 168-171 and 176 were achieved for any panel of sample."

Applicants respectfully submit that *all* of the examples and figures included with the application represent *selected* panels and demonstrate the limitations of claims 168-171. It is a prerequisite of the claimed method that primer-probe sets are identified that result in amplification efficiencies within the limits claimed. Therefore, none of the examples provided or figures included lie outside those limitations.

The second point to be addressed regards the Examiner's reference to the Erlander et al WO application. The very fact that the Examiner has provided, for comparison, a reference disclosing "coefficient of variations on the order of 15% or greater" supports the Applicants' position that the presently claimed invention is novel and unobvious. The coefficients of

variation with respect to reproducibility cited in Erlander at al. represent the state of the art prior to the presently claimed invention. Until the presently claimed invention, such low coefficients of variation were unprecedented. Yet, surprisingly, Applicants have succeeded in devising a system for achieving reliable and reproducible amplification efficiencies with the heretofore unheard of coefficients of variation as low as required by the limitations of claims 168-171.

The third point is that the Examiner found no references that discussed determining a coefficient of variation in reproducibility any lower or in the range of the claimed limitation of 3%. Applicants are not sure if by "references" the Examiner means prior art references, or references within the application. If the Examiner means references within the application, Applicants respectfully draw the Examiner's attention to p. 23, lines 6-33, particularly lines 13-15 (... *we found* an average coefficient of variation ... of less than 1 percent...) and lines 18-19 (... *we found* an average coefficient of variation of less than 2 percent (emphasis added). If, however, the Examiner means prior art references, then it is believed that the Examiner will not find any references that discuss determining a coefficient of variation any lower or in the range of the claimed limitation of 3%, because Applicants are believed to be the first to reproducibly achieve such coefficients of variation. Applicants respectfully submit that it is not possible to fully understand the enormity and novelty of the presently claimed invention without realizing this important distinction from what has been done before by others in the field.

Evaluation of a biological condition

The Office Action asserts that because claims 167 and 175 are directed to "a method for evaluating a biological condition of a subject" and that the only positive step in these claims is to "derive a profile data set from a sample" that the methods are not enabled for evaluating a biological condition because there is no step that results in evaluating a biological condition. See

Office Action, p. 5, first para.

Applicants respectfully submit that the profile data set which results from following the method of claims 167 and 175 is itself an evaluation of the biological condition. It should be remembered that these claims require that the panel of constituents is "selected so that measurement of the constituents enables evaluation of a biological condition." As stated by Dr. Tryon, "once a profile data set has been obtained, it is necessarily the case that a biological condition has been evaluated." Declaration, para. 21.

This surprising result is shown by a variety of examples in the application. For example, "Figures 11(a) and 11(b) show different inflammatory stimuli that give rise to different, baseline profile data sets that may be used in determining the calibrated selected profile data sets ..., and the resulting different signature profiles." See specification, p. 47, lines 3-6. And further, "Figure 11(a) also illustrates the extraordinary range of detection (y-axis) from less than 10 fold difference from the calibrated profile with respect to some constituents to a change of 10^{13} ... when compared to the calibrator. Comparison to the calibrator results in gene expression profiles that are increased, decreased, or without change from the calibrated set." *Id.*, lines 8-14.

Similarly, each of Figs. 19a and 19b (see discussion at p. 14, lines 20-27) compare gene expression profiles for a subject in relation to a normal subject. In Fig. 19(a), the subject is a smoker and the subject's difference physiological condition is evaluated by the changes indicated above and below baseline as shown. In Fig. 19(b), the subject suffers from chronic obstructive pulmonary disease, and the subject's different physiological condition is evaluated by the changes indicated above and below baseline as shown. The term used for the pattern of expression in an expression profile with suitably selected genes, is a diagnostic "signature" panel. See p. 14, lines 23-24; also p. 18, lines 25-29 (definition of "signature profile" and

"signature panel") as well as p. 19, lines 9-11 (use thereof for describing a biological condition or an effect of an agent on a biological condition) and p. 30, lines 12-13 and 28-30 (evaluating activity of an agent). These concepts are discussed at length on p. 24, lines 5-24.

Indeed, the specification clearly states that "Given the precision we have achieved in measurement of gene expression, described above and in connection with selected panels and gene amplification, we conclude that where differences occur in measurement under such conditions, the differences are attributable to difference in biological condition." See p. 29, lines 3-6. And, the description goes on to elaborate how to use a calibrated profile data set to evaluate a biological condition.

In the present case, the specification describes how to select a panel of genes to be used for deriving a profile data set and how to obtain such measures required to derive the profile data set (see p. 22, line 4 through p. 25, line 8) by strictly adhering to a narrow permissible level of amplification efficiencies (p. 25, line 9 through p. 26, line 16). The claimed data set, as discussed above, provides evaluation of a biological condition in a manner analogous to a subject's body temperature. Dr. Tryon points out that "taking someone's temperature allows evaluation of the presence or absence of a fever based on the number measured by the thermometer." Declaration, para. 21. The mere act of taking a temperature – i.e. obtaining a measurement that is accurate and reproducible - is a method for evaluating a biological condition because the number itself allows the person taking the temperature to assess whether there is a fever in the subject just by knowing what that number is.

Similarly, as elaborated by Dr. Tryon, "taking someone's blood pressure, or heart rate, or blood glucose level, or performing countless other diagnostic measurements, allows one skilled in medicine to evaluate a biological condition. Any of the above measurements, obtained by

someone lacking in medical training is simply a number. To those skilled in the art of medicine, however, a deviation in someone's blood pressure level, glucose level, or heart rate, for example, facilitates evaluation of a biological condition, whether it be anxiety, arterial bleeding, arteriosclerosis, diabetes, or cardiacarrhythmia.." Declaration, para. 22.

In the present case, the claimed method of deriving a profile data set, which includes a plurality of quantitative measures, and, in deriving the profile data set, obtaining such measure under conditions that are substantially reproducible, allows evaluation of a biological condition according to the nature of the individual profile data set obtained. As shown in the examples discussed above, one trained in medicine can evaluate the presence or absence of a biological condition merely by seeing the nature of the profile data set obtained for a patient, just as would be done by seeing the temperature, blood pressure, glucose level, or heart rate of that patient.

According to Dr. Tryon, "although taking someone's temperature, or blood pressure, or blood glucose level or heart rate is a single measurement, the presently claimed invention is analogous in that a matrix of measurements is used to evaluate a biological condition, rather than a single measurement (providing more data, in a manner like using blood pressure, temperature, and heart rate in combination.)" Declaration, para.23. Therefore, for the reasons stated above, Applicants respectfully submit that the claims are enabled for providing the required result of the preamble – evaluating a biological condition.

Amplification of proteins

The instant application claims "A method for evaluating a biological condition of a subject... comprising: deriving from the sample a profile data set, ... each member being a quantitative measure of the amount of distinct RNA *or protein* constituent in a panel of constituents....; and in deriving... obtaining such measure for each constituent under

measurement conditions that are substantially reproducible...." See claim 167, emphasis added. According to the Office Action, however, "For those members ... that are proteins, the specification does not disclose amplification of protein or efficiencies of amplification therefore. Likewise, no specificities (of amplification?) are disclosed with respect to proteins.... The specification does not disclose nor provide guidance to measuring protein levels in these ways." See Office Action, p. 5, 2nd para.

First, Applicants respectfully submit that the specification *does* disclose amplification of proteins. As pointed out on p. 27, lines 13-16, "The figures provided here are directed to RNA. However, methods herein may also be applied using proteins where sensitive quantitative techniques [for amplification], such as an Enzyme Linked ImmunoSorbent Assay (ELISA), are available and well-known in the art for measuring the amount of a protein constituent."

Second, regarding amplification specificity of proteins, and calibration of protein expression, as explained by Dr. Tryon, "The concepts of precision, specificity and calibration of mRNA are not limited to just mRNA. As defined in the application, protein, like RNA, is a gene expression product." Declaration, para. 24. Further, the limitations of the claimed methods are not, as assumed by the Examiner, "only applicable to quantitative measures of RNA" see Office Action, p. 5, 2nd para. Those skilled in the art would readily understand the transferability of the principles controlling specificity and efficiency of amplification of mRNA, and the principles controlling the ability to quantitate mRNA amplification, to the principles for controlling specificity, efficiency, and the ability to quantitate expression of proteins using ELISA. As relayed by Dr. Tryon, "State-of-the-art methods for quantifying expression products, whether RNA or protein, are remarkably similar at the physical (quantum) level." Declaration, para. 27. Elaborating on that concept, Dr. Tryon notes that "this commonality has been appreciated for

decades by those trained in physical biochemistry (see, for example, Friedlander, et al.) but has been reinforced more recently by the publication of methods for the induced replication of prions (infectable proteins) that have been described as analogous to gene amplification by PCR (see for example Saborio, G.P. et al., *Nature* (2001) 422:810-813; Soto, C., et al., *Trends in Neurosciences* (2002) 25:390-394; and Telling, G., Protein-based PCR for Prion Disease?, *Nature Medicine* (2001) 7:778-779; Declaration, para. 28).

The presently claimed invention emphasizes the requirement for precision, reproducibility and calibration to accurately describe a biological condition using RT-PCR technology. As stated in the specification on. p. 27, lines 13-16, quantitation of protein expression using ELISA would result in an accurate description of a biological condition based on a selected panel of proteins. Dr. Tryon emphasizes that "The fundamental concept of an ELISA is that there is a definable ratio between the number of protein molecules expressed and the number of antibodies bound. In the practical world, the *absolute* number of bound antibodies is impossible to determine." See Declaration, para. 30.

To address this limitation, scientists have developed a way to amplify the signal of the number of proteins expressed in any given system so they can control amplification efficiency, specificity, and the ability to quantitate the amount of protein expressed from any given gene. Thus, according to Dr. Tryon, and as readily understood by those skilled in the art, "a detectable (quantitatable) reporter molecule or enzyme is added to the antibody. There is now a definable ratio between the quantity of proteins and the number of detectable (quantitatable) reporter events. The reporter molecule amplifies the signal. The signal is equal to the quantity of antibodies bound to the cognate protein." *Id.*

In truth, mRNA produced in PCR reactions must also be amplified to be detectable and

quantitatable. This makes control of amplification efficiency and specificity, and the ability to quantitate mRNA produced in PCR systems analogous to these same issues in protein expressions using ELISA. According to Dr. Tryon, "In the practical world, neither the antibody signal in the ELISA nor the probe signal in quantitative PCR is detectable without additional amplification. Signal is amplified in the ELISA by adding additional reporter-bearing antibodies (i.e., the more antibodies, the more signal generated). Signal is amplified in quantitative PCR by replicating the number of double-stranded products that incorporate signal (i.e., the more products, the more signal). Declaration, para. 32.

In Dr. Tryon's expert opinion, "the claimed invention in the present application shows how to produce gene-product-to-gene-product calibration by providing guidance in how to control the efficiency of the signal amplification process, and those skilled in the art would understand how this works in the context of protein expression or mRNA production. Since the invention of ELISA and PCR, guidance for increasing specificity has been provided in thousands of journal articles and hundreds of protocol manuals. That is, understanding control over specificity is a minimal requirement to be perceived as skilled in the art." See Declaration, para. 34.

Applicants respectfully submit that those skilled in the art would know and understand these similarities and transferability of principles between amplification of mRNA and amplification of proteins. Further, such information is readily available to those skilled in the art through scholarly journal articles, reviews, and books and "How-To Manuals" for performing ELISA. As such, Applicants respectfully submit that the limitations of the presently claimed invention *are a*pplicable to measuring protein levels as well as mRNA levels and that the application as written is enabled for preparation of calibrated and baseline profile data sets,

signature profiles and data sets, and evaluation of a biological condition based on control of amplification efficiency, specificity, and the ability to quantitate both mRNA production by PCR and protein expression by ELISA.

Undue experimentation to practice invention

Regarding the *In re Wands* factors and the Examiner's assertion that "The specification acknowledges that a large amount of experimentation would be required to develop the conditions.... " (see Office action, p. 6, second full para.), Applicants are unable to find the section in the application that makes such an acknowledgment and the Examiner has not provided the cited page. In fact, the specification states the exact opposite. For example, on p. 21, lines 29-32 it states: "Nonetheless, the methods disclosed here may be applied to cells of other organisms without the need for undue experimentation by one of ordinary skill in the art because all cells transcribe RNA and it is known in the art how to extract RNA from all types of cells."

As stated in the Manual of Patent Examining Procedure (MPEP) sec. 2164.01, "The fact that experimentation may be complex does not necessarily make it undue, if the art typically engages in such experimentation." quoting from *In re Certain Limited-Charge Cell Culture Microcarriers*, 221 USPQ 1165, 1174 (Int'l Trade Commm'n 1983) and also *In re Wands*, 858 F.2d at 737. In the present case, "it has historically been, and continues to be, standard operating procedure in laboratories practicing PCR amplification today that determination of amplification is experimentally derived or confirmed. That is, the invention requires some *minimal* experimentation." Declaration, para. 35, emphasis added.

Further, as pointed out by Dr. Tryon, a person of at least ordinary skill in the relevant art, " ...common practice for every skilled laboratory applying RT-PCR to human specimens, and

recommended in every reference cited above, is to overlay a system of quality control or design rules for potential primer-probes sets. For example, the primer-probe sets have to yield a single band of the expected size as observed by gel electrophoresis and the sequence of the amplified product have to be identical to the expected product." Declaration, para. 36. In fact, the authors of the reference cited by the Examiner do precisely such a control (see Rodriguez-Antona et al., Fig. 2 and last para. of p. 110 through first 2 lines of p. 111 – "...all amplified CYPs yielded single bands of the expected size....").

In the present case, "When these rules were applied with the design rules specified in the application and combined with the narrow requirements for amplification efficiency specified in the application, each primer-probe set met the requirements for the invention, i.e., each primer-probe set had high reproducibility (precision) and exhibited a calibrated or reproducible, ratiometric relationship to other assayed constituents in the selected panel, *in every case tested.*" Declaration, para. 37.

Further, according to Dr. Tryon and as discussed above regarding design and experimental verification of primer/probe sets, "even if a sequence at a different region of the same locus were chosen, the possible primer-probe combinations could be assessed with minimal experimentation and more than one primer-probe could set meet the conditions required for the invention; i.e. specificity and amplification efficiency. Declaration, para. 38.

The Office Action states, however, that "In order to practice the claimed methods, the skilled practitioner would first turn to the instant specification for guidance ... [then] to the prior art for such guidance, ... [then] to trial and error experimentation ... without guidance ... For all these reasons, it is considered to require undue experimentation to practice the method of the claims." See Office Action, p. 7, 4th para.

As described above at length, the specification provides enough specific guidance, when coupled with the knowledge generally available to one skilled in the art regarding primer-probe design and selection, to practice the method of the claims without resorting to the abyss of blind trial and error experimentation, or experimentation at levels high enough to fall into the very nebulous category of "undue experimentation." Manuals provided with commercial PCR instruments, books available in any university library, scholarly journal articles and reviews dedicated to the design of primer-probe sets for PCR amplification – all such forms of knowledge in design of PCR primer-probes are readily available to those skilled in the art with minimal effort and expense. Coupled with the requirements in the instant specification for design of primer-probe sets having a very narrow limit in coefficient of variation of amplification efficiency, (which clearly states that primer/template ratio is an additional factor beyond the Siebert factors affecting amplification efficiency) and finally combined with the guidance in the specification for what those primer/template ratios should be (maintained for all assayed loci within a 10-fold range), and what the required coefficients of variation must be (differing by no more than 10%, 2% or 1%), Applicants respectfully submit that a person of average skill in the art would have more than enough guidance to practice the method of the invention without the need to perform undue experimentation.

In summary, Applicants respectfully submit that as affirmed by Dr. Tryon, "those skilled in the art typically engage in the design and selection of primer-probes sets, they typically engage in experimental verification that the primer-probes sets so-designed and identified actually work as desired/required, and they typically engage in efforts to achieve the most efficient amplification technically feasible, which is determined experimentally. They also typically engage in screening very large numbers of genes from various organisms to develop

gene expression profiles, as indicated by the enormous amount of literature on the subject. For example, when the term "gene expression profile" was plugged into the patent section of the USPTO web site by Applicants, as of May 5, 2003, such a search yielded 66 patents for the years 1976 to the present, and 305 patent applications for the years 2001 – 2003 alone!" (see Declaration, para. 39), the only years for which patent applications have been published.

Applicants respectfully submit that those skilled in the art are regularly practicing the skills necessary to obtain gene expression profiles such as disclosed in the presently claimed invention. In the present case however, because Applicants are the first to realize and understand the importance of primer-template ratios on amplification efficiency, Applicants obtain gene expression profiles while also requiring, and more importantly reproducibly achieving, amplification efficiencies with coefficients of variation which differ by no more than 10%, 2% and 1%, something not accomplished until the presently claimed invention.

37 CFR § 102(b)Rejections

THE SUBJECT MATTER DEFINED BY THE CLAIMS IS NOT DISCLOSED OR TAUGHT BY THE PRIOR ART OF RECORD

As required by the U.S. Patent and Trademark Office, "A claim is anticipated only if each and every element as set forth in the claim is found, either expressly or inherently described, in a single prior art reference." See MPEP § 2131, quoting *Verdegaal Bros. v. Union Oil Co. of California*, 814 F.2d 628, 631 (Fed. Cir. 1987). Applicants respectfully submit that the cited reference Rodriguez-Antona et al. does not anticipate any of the claims of the instant application, nor does it render the presently claimed invention obvious.

As a preliminary matter, it is useful to note that independent claims 167 and 175 require,

among other things, that a single set of measurement conditions must be maintained for *all* constituents in the panel. These measurement conditions are at a minimum that, for all constituents, the measurement conditions must be substantially reproducible. In implementing the systemic requirements of these claims, the application notes that it is concluded "where differences occur in measurement under such conditions, the differences are attributable to differences in biological condition," p. 29, lines 3-6. The systemic approach, defined by the claims herein, distinguishes the subject matter herein from the prior art.

In the present application, the claims require measurement conditions that are substantially reproducible for each constituent in a profile data set (claims 167 and 175), and require efficiencies of amplification for all constituents that are substantially similar (claim 167) such that they differ by no more than 10%, 2% and 1% (claims 169, 170, and 171, respectively). The Office Action states that "The primers used were designed so that specificity and efficiency of amplification were similar. Reproducibility of the assay was determined." *See* Office Action p. 8, middle of last para. Applicants respectfully submit, however, that this is not what Rodriquez-Antona et al. says about how their primers were designed. According to Rodriquez-Antona et al., "The primers were selected in order to meet three [sic] requirements: (a) to have similar melting temperatures, (b) to match specific, nonconserved regions of each CYP, (c) to render amplified cDNA fragments of similar sizes, and (d) to flank intronic sequences ... " See Rodriquez-Antona et al., p. 110, 2nd col., first para. of Results. Later, Rodriquez-Antona et al. state that to achieve the aim of the study (reliable quantitative RT-PCR assay in one single PCR run) "... primers (Table I) and amplification conditions were selected to assure the simultaneous exponential amplification of the selected genes and to produce unique amplification products." Applicants respectfully point out that assurance of "simultaneous exponential amplification of

the selected genes" is not the same thing as having efficiencies of amplification that are substantially similar, or measurement constituents for each constituent that are substantially reproducible.

First, nowhere in Rodriquez-Antona et al. is there any mention of designing primers, or an overall system, wherein actual amplification efficiencies are required, and experimentally verified, to differ by no more than 10% (3%) (1%). Any mention in Rodriguez-Antona et al. regarding efficiency is always in relation to reverse transcriptase efficiency, not amplification efficiency (which is only estimated in Rodriguez-Antona et al.).

Also, there is no teaching in Rodriguez-Antona et al. with regard to actually measured and verified amplification efficiencies. Rodriguez-Antona et al. have developed a quantitative RT-PCR assay that relies on normalization of reverse transcription in the RT step by the use of a luciferase mRNA standard, followed by *estimation* of the degree of amplification of cDNA in the PCR step using appropriate cDNA standards (see Rodriguez-Antona et al., p. 109, col. 1, emphasis added). The Rodriguez-Antona et al. RT-PCR assay has optimized *efficiency of mRNA reverse transcription* in the RT step until it is "highly specific, reproducible, rapid and sensitive enough to quantitate low and high abundant mRNAs." *See id.*, lines 4-6. In the second step, the PCR step, Rodriguez-Antona et al. use cDNA standards to generate a standard cDNA amplification curve, from which the amplification efficiency of the sample cDNA is *estimated.* Rodriguez-Antona et al. explain that "As schematized in Fig. 1 three different amounts of each of the CYP standards and appropriate dilutions of the sample to be tested were used in each PCR assay ... The concentration of specific cDNAs in samples *was estimated* from the amount of amplified product measured and the amplification ratio (cDNA content of standards before and after PCR amplification)" (see Rodriguez-Antona et al., p. 110, col. 2, 2nd and 4th full paragraphs,

emphasis added).

In contrast, the presently claimed invention requires *cDNA amplification efficiencies* that differ by no more than 10%, 2%, and 1%, respectively (for example, in claims 169, 170 and 171, respectively, and see specification, p. 26, lines 8-16), achieved by limiting primer-template ratios to within a 10-fold range (*id.*) and by experimental verification. The presently claimed invention does not rely on cDNA standards to estimate cDNA amplification efficiencies of samples being tested, but actually measures the amplification efficiency of each sample and confirms that it is within the required range before using that data point to derive a profile data set.

Further, the Office Action states that "With respect to claims 168 and 176, Fig. 5 ... and p. 112 ... demonstrate repeated testing showing little intraassay variability..." (see Office Action, bot. p. 8 through top of p. 9). Applicants respectfully draw the Examiner's attention to the fact that such data and discussion in Rodriquez-Antona et al. relates to *reverse transcription* efficiency, not *cDNA amplification* efficiency. Applicants respectfully remind the Examiner that the intraassay variability and interassay variability of concentration measurements described in the presently claimed invention (see specification, p. 23, lines 10-20) are based on narrow ranges of actual amplification efficiencies experimentally verified and does *not* refer to variability in efficiencies of reverse transcription. Thus, to the extent that the Rodriquez-Antona et al. reference deals with efficiency of reverse transcription of mRNA, it is not relevant as prior art. Dr. Tryon points out that "reproducibility or efficiency of the reverse transcriptase step: (1) is not the same as amplification efficiency (as used in the application or the established art); (2) was well established in the art prior to submission of this application for uses similar to those in this application; and (3) is misapplied as germane to the question of anticipation of the presently claimed invention." Declaration, para. 40.

As there is no disclosure in the cited reference whatsoever of control of amplification efficiencies, there can be no anticipation of claim 167, which requires that efficiency of amplification for all constituents be "substantially similar," nor of any claim depending from claim 167. In addition to the absence of any disclosure regarding control of efficiency of amplification, there is no teaching in the reference of such control either. For this reason, the subject matter of claim 167 and claims depending from 167 would not have been obvious in light of the Rodriquez-Antona et al. reference.

Second, and perhaps more importantly, independent claims 167 and 175 also require that the relevant measurement conditions be "substantially reproducible." Despite use in the reference of the word "reproducible" (see Rodriquez-Antona et al., Abstract and p. 115, col. 1, for example), in fact, Rodriquez-Antona et al. fail to disclose or suggest measurement conditions that are "substantially reproducible." Although Table III[1] in the reference provides the best argument that the reference discloses conditions that are "substantially reproducible," a careful reading of the reference shows that its disclosure falls far short of this. First, as explained by Dr. Tryon, "the values depicted in Table III are not direct measurements but are rather ratios of molecules of mRNA/microgram of total RNA" (see Declaration, para. 41 and Rodriquez-Antona et al., p. 115, Table III). In turn, according to Dr. Tryon, "it appears that neither the numerator nor the denominator of this fraction is directly measured either, but rather both are the result of calculation and estimation. The numerator, number of molecules of mRNA, is determined by reference to a luciferase mRNA standard (see Rodriquez-Antona et al., p. 115, col. 1, 1st para.). The denominator appears to be derived from estimation as well. *Id.*, and also see Table II of Rodriguez-Antona et al..

Moreover, Dr. Tryon notes that "the variation listed in the individual derived values in Table III cannot be evaluated because no experimental details are provided. Declaration, para. 42. In particular, "a calculated value of (1100 ± 100) x 10^5 for 2C9 does not tell us whether the error is the authors' estimate of accuracy of a single calculation or whether some number of determinations and calculations were made, and how variation of this sampling is reported." *Id.*

Dr. Tryon continues, pointing out that "there are more problems yet with Table III. We see results for four different liver samples for which no different physical or biological condition is identified, and the variation from one sample to the next runs from about 300% to over 1000%." *Id.* These apparent interassay variabilities are orders of magnitude higher than the requirement for measurement conditions that are "substantially reproducible" in the subject matter claimed herein.

Further, according to Dr. Tryon, "the large variability demonstrated in Table III of the reference is substantiated by the authors' own characterization of their results as being "in agreement with" results of previous experimenters who achieved results such as 3.4 ±2.4 for 1A2 (variation of 71%), 1.8 ± 1.9 for 2A6/7 (variation of 106%), etc." *Id.*

Also, claims 167 and 175 require that the quantitative measure of the amount of distinct RNA or protein constituent be obtained "*for each constituent* under measurement conditions that are substantially reproducible." See claims 167 and 175, emphasis added. Table III of Rodriguez-Antona et al. does not meet such strict requirements. Even if one were to assume that the values in Table III represent actual measurements (which they do not), Table III discloses that measurement conditions for all constituents in the Rodriguez-Antona et al. system can vary widely, and this appears to be acceptable to the authors. As stated by Dr. Tryon, "Simple

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[1] Apparently referred to inadvertently by the Examiner as Table I

observation of the values in Table III shows variances of 45-fold (4500%) or greater for gene 2E1; 20-fold (2000%) or greater for gene 1A1; and 10-fold (1000%) or greater for genes 2C9, 3A4, and 3A5; etc. Further, Rodriguez-Antona et al claim that these results are similar to other published results for the same genes which show variations of 100% or more. Such extremely large fluctuations in interassay variability are totally unacceptable in the presently claimed system, which requires that the measurement of the constituents in the panel be obtained for each constituent under measurement conditions that are substantially reproducible." Declaration, para. 43.

In short, Rodriguez-Antona et al. discloses nothing by way of measurement conditions that are "substantially reproducible" in the manner required by the claims now pending, whether for amplification efficiencies or for the measurements obtained for each constituent. Nor does the reference suggest any means by which "substantially reproducible" measurements could be achieved. In fact, the widely varying results disclosed in the reference highlight the non-obviousness nature of the subject matter claimed here.

Lastly, the application claims priority to June 28, 1999. At that time, the state of the art relevant to the claimed subject matter is well represented by five references that were filed with a supplemental Information Disclosure Statement on December 23, 2002, designated CV-CZ. None of these references anticipates the presently claimed invention, nor suggests modifying any of the disclosed systems to arrive at the subject matter claimed herein. As summarized by Dr. Tryon "All these references, which reflect the state of the art, disclose a measurement technique in which the coefficients of variation are calculated only in relation to a ratio between an experimental target and a standard. No information is provided as to the actual coefficient of variation for repeated measurements of the same sample." (see Declaration, para. 44).

The subject matter claimed herein requires determination of coefficients of variation for repeated actual measurements of the same sample, something completely novel relative to the state of the art at that time. According to Dr. Tryon, "reference CZ regards its technique of providing an ability to detect with confidence changes of 20% in mRNA copy number as novel (see Zhang et al., p. 347, col. 1, first full para.). Moreover, this same reference teaches that restricting the range of amplification even within as much as 10% is undesirable because it results in exclusion of data (*id.*, p. 345, beginning of col. 2)" (see Declaration, para. 45). However, as discussed above, when the requirements for amplification efficiency and coefficients of variation of the presently claimed invention are met, *any* change in measurement of mRNA expression is taken to indicate a change in biological condition (see above, p. 33 and p.42, and specification, p. 29, lines 3-6).

In summary, the claims, as amended have support in the specification and do not contain new matter. The claims are enabled for someone of ordinary skill in the art for the design and preparation of primer/probe sets that meet the reproducibility and amplification efficiency requirements of the presently claimed invention, for amplification of proteins as well as mRNA, for evaluation of a biological condition, and the specification, as written, does not require undue experimentation to practice the invention.

The cited reference, Rodriguez-Antona et al., does not contain every element of the claims and so does not anticipate the presently claimed invention. In particular, Rodriguez-Antona et al. do not disclose measurement conditions for each constituent that are substantially similar, nor do they disclose efficiencies of amplification that are substantially reproducible, differing by no more than 10%, 2% and 1%. Moreover, the cited reference does not teach these elements either, and so does not render the present claims obvious. Nothing in Rodriguez-

Antona et al. suggests how one would modify the disclosed system, whether alone or in combination with knowledge generally available in the art, to arrive at the substantially similar measurement conditions for each constituent or arrive at the substantially similar efficiencies of amplification required by the presently claimed invention.

CONCLUSION

For the reasons set forth above, it is submitted that all pending claims are in condition for allowance. Reconsideration of the claims and a notice of allowance are therefore requested.

Applicants respectfully request a two-month extension of time; however, this conditional petition for an additional extension of time is being made in the event that the need for an additional extension has been overlooked. Please pay any fees required for the timely consideration of this application from deposit account number 19-4972. The Examiner is requested to telephone the undersigned if any matters remain outstanding so that they may be resolved expeditiously.

May 27, 2003

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